

## **Supplementary Information**

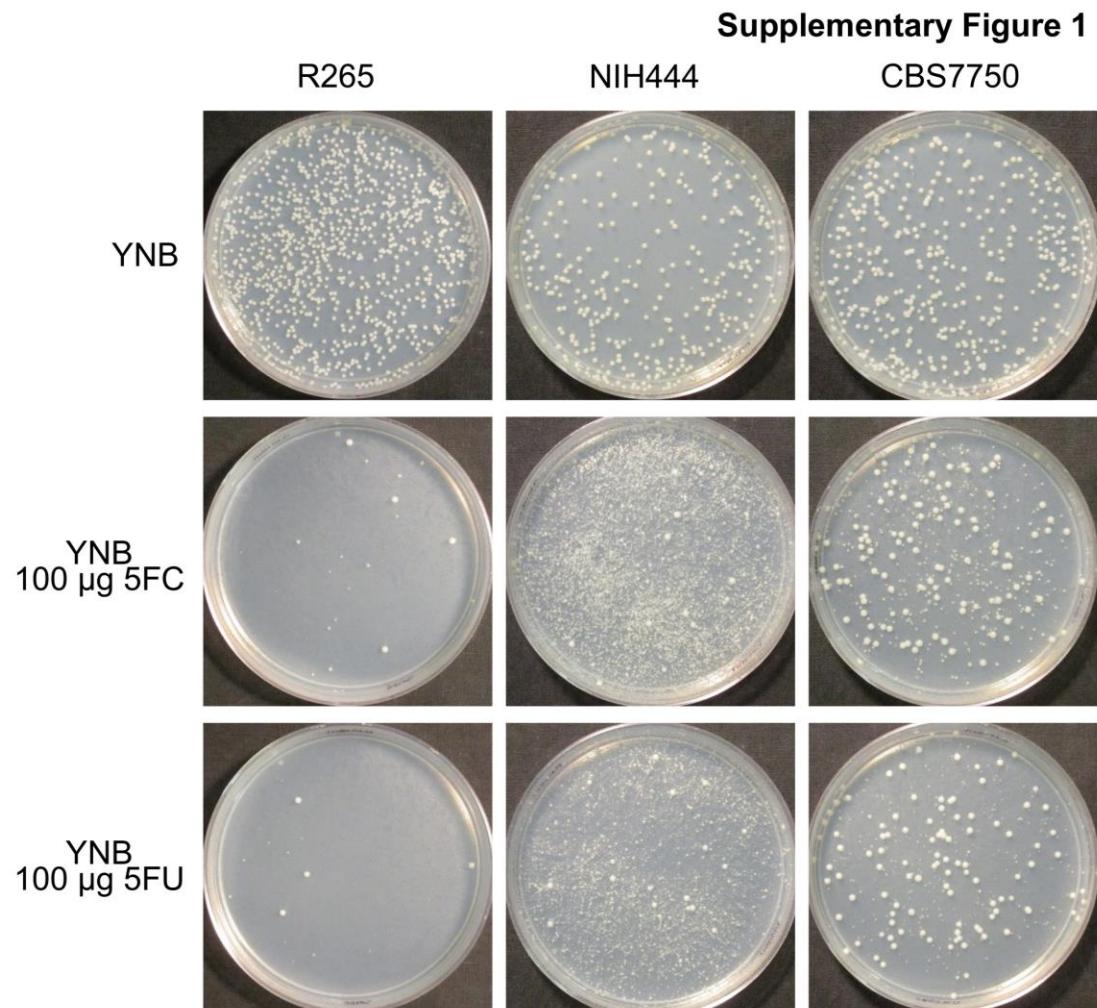
### **Hypermutation in *Cryptococcus* reveals alterations in capsule biosynthesis are associated with 5-fluorocytosine resistance**

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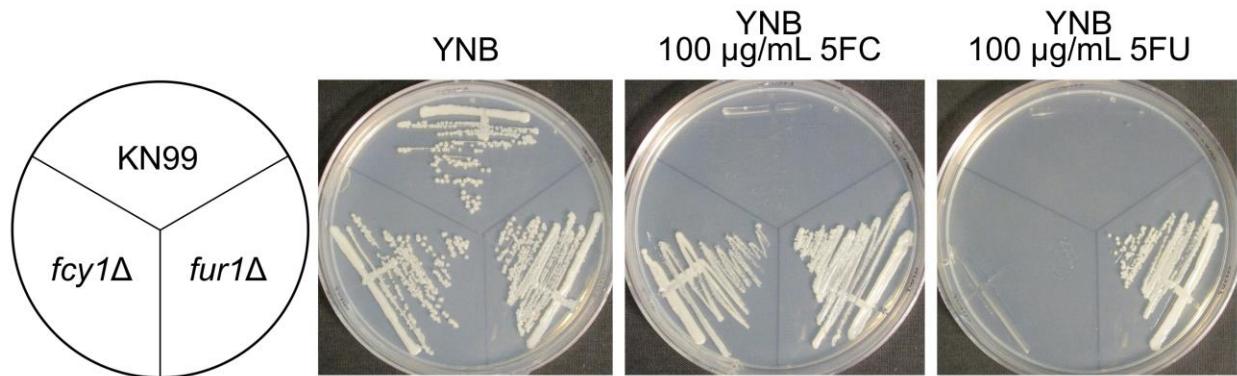
**Supplementary Figure 1. VGIIa-like isolates acquire resistance to 5FC and 5FU more rapidly than the VGIIa isolate R265.**



VGIIa-like strains NIH444 and CBS7750 that harbor *msh2* nonsense alleles were tested for the ability to generate resistance to 5FC and 5FU in comparison with the closely related VGIIa strain R265. For each strain, 5 mL YPD cultures were inoculated from a single colony and grown overnight at 30°C. After washing, 100 µl of a 10<sup>-5</sup> dilution was plated to YNB control plates and 100 µl of undiluted cultures was plated on media containing 5FC or 5FU. The VGIIa-like strains generated substantially more isolates resistant to both drugs.

**Supplementary Figure 2. Mutants of *fcy1* and *fur1* in *Cryptococcus neoformans* are resistant to 5FC but not 5FU.**

**Supplementary Figure 2**



*fur1 $\Delta$*  and *fcy1 $\Delta$*  strains from the KN99 *C. neoformans* collection were struck onto YNB, YNB + 100  $\mu$ g/mL 5FC, and YNB + 100  $\mu$ g/mL 5FU. While the *fcy1 $\Delta$*  mutant strain grew on media containing 5FC, it did not grow on media containing 5FU. In contrast, the *fur1 $\Delta$*  mutant strain grew on media with either drug.

**Supplementary Table 1.** Strains and plasmids used in this study

Strain name	Genotype	Construction or source
RBB17	R265 <i>MATα msh2Δ::NEO</i>	Billmyre et al, 2017 <sup>1</sup>
RBB18	R265 <i>MATα msh2Δ::NEO</i>	Billmyre et al, 2017 <sup>1</sup>
SEC612	KN99 <i>MATα ugd1Δ::NEO</i>	Biolytic transformation
SEC613	H99 <i>MATα ugd1Δ::NEO</i>	SEC612 x SEC615
SEC614	KN99 <i>MATα uxs1Δ::NAT</i>	KN99 <sup>a</sup> x KN99 <sup>a</sup> <i>uxs1Δ::NAT</i>
SEC615	H99/KN99 <i>MATα uxs1Δ::NAT</i>	H99 x SEC614
SEC616	KN99 <i>MATα ugd1Δ::NEO uxs1Δ::NAT</i>	SEC612 x SEC615
SEC617	H99 <i>MATα ugd1Δ::NEO uxs1Δ::NAT-1</i>	SEC612 x SEC615
SEC618	H99 <i>MATα ugd1Δ::NEO uxs1Δ::NAT-2</i>	SEC612 x SEC615
TDY1787	KN99 <i>MATα uxs1Δ::NAT</i>	Li et al, 2018 <sup>2</sup>
TDY1811	KN99 <i>MATα uxs1Δ::NAT UXS1::NEO</i>	Li et al, 2018 <sup>2</sup>
TDY1799	KN99 <i>MATα P<sub>ACT1</sub> UXS1</i> overexpression ( <i>NAT</i> )	Gish et al, 2016 <sup>3</sup>
TDY1679	KN99 <i>MATα uxt1Δ::NEO</i>	Li et al, 2018 <sup>2</sup>
TDY1685	KN99 <i>MATα uxt2Δ::NAT</i>	Li et al, 2018 <sup>2</sup>
TDY1695	KN99 <i>MATα uxt1Δ::NEO uxt2Δ::NAT</i>	Li et al, 2018 <sup>2</sup>
TDY1076	KN99 <i>MATα cxt1Δ::NAT</i>	Klutts et al, 2008 <sup>4</sup>
TDY1077	KN99 <i>MATα cxt2Δ::NEO</i>	Klutts et al, in preparation
TDY1078	KN99 <i>MATα cxt1Δ::NAT cxt2Δ::NEO</i>	Klutts et al, in preparation
	KN99 <i>MATα fur1Δ::NAT</i>	Madhani collection
	KN99 <i>MATα uxs1Δ::NAT</i>	Madhani collection
	KN99 <i>MATα fcylΔ::NAT</i>	Madhani collection
	KN99 <i>MATα fcyl2Δ::NAT</i>	Madhani collection

**Supplementary Table 2.** Oligonucleotides used in this study

Primer	Sequence	Description
JOHE45233	gtaacgccagggtttcccagtcacgacgCCAAA TGTGTTGCTATGTG	5' primer to amplify 1 kb upstream <i>UGD1</i> for homologous recombination gene deletion. Includes homology to pGI3.
JOHE45085	ctggccgttgtttaTTTGAATGGGGTTG AGGGTA	3' primer to amplify 1 kb upstream <i>UGD1</i> for homologous recombination gene deletion. Includes homology to <i>NEO</i> .
JOHE45086	TACCCTCAACCCCATTCAAAtaaaa cgacggccag	5' primer to amplify <i>NEO</i> for homologous recombination gene deletion of <i>UGD1</i> . Includes homology to <i>UGD1</i> upstream region.
JOHE45087	GTCGCCGGTACCGATAAGTcaggaaa cagctatgac	3' primer to amplify <i>NEO</i> for homologous recombination gene deletion of <i>UGD1</i> . Includes homology to <i>UGD1</i> downstream region.
JOHE45088	gtcatagctgttcctgACTATCGGTACC GGCGAC	5' primer to amplify 1 kb downstream <i>UGD1</i> for homologous recombination gene deletion. Includes homology to <i>NEO</i> .
JOHE45234	gcggataacaattcacacagaaacagcCTC ACGATTGCCTCATAAAC	3' primer to amplify 1 kb downstream <i>UGD1</i> for homologous recombination gene deletion. Includes homology to pGI3.
JOHE45303	GCGTTGAAGTGGTAAGTG	Internal 5' <i>UGD1</i> screening primer
JOHE45304	GACGATCTTGGAAAGAGGTAG	Internal 3' <i>UGD1</i> screening primer
JOHE45335	GTCCTCGACAACCTCTTCAC	Internal 5' <i>UXS1</i> screening primer
JOHE45336	CGGTGATAACCATAGGTC	Internal 3' <i>UXS1</i> screening primer
JOHE41579	CTAACTCTACTACACCTCACGGCA	5' <i>STE20α</i> screening primer
JOHE41580	CGCACTGCAAAATAGATAAGTCTG	3' <i>STE20α</i> screening primer
JOHE41581	GGCTGCAATCACAGCACCTTAC	5' <i>STE20α</i> screening primer
JOHE41582	CTTCATGACATCACTCCCCTAT	3' <i>STE20α</i> screening primer

## Supplementary References

1. Billmyre, R. B., Clancey, S. A. & Heitman, J. Natural mismatch repair mutations mediate phenotypic diversity and drug resistance in *Cryptococcus deuterogattii*. *eLife* **6**, e28802 (2017).
2. Li, L. X., Rautengarten, C., Heazlewood, J. L. & Doering, T. L. Xylose donor transport is critical for fungal virulence. *PLoS Pathog.* **14**, e1006765 (2018).
3. Gish, S. R. *et al.* Computational analysis reveals a key regulator of cryptococcal virulence and determinant of host response. *mBio* **7**, e00313-16 (2016).
4. Klutts, J. S. & Doering, T. L. Cryptococcal xylosyltransferase 1 (Cxt1p) from *Cryptococcus neoformans* plays a direct role in the synthesis of capsule polysaccharides. *J. Biol. Chem.* **283**, 14327–14334 (2008).